RESEARCH ARTICLE

ANTITUSSIVE EVALUATION OF FORMULATED POLYHERBAL COUGH SYRUP

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ABSTRACT

Ethanolic extracts of fruits of *Terminalia chebula (Combretaceae), leaves of Mentha piperita (Labiate),* leaves of *Adhatoda vasica (Acanthaceae),* leaves of *Ocimum sanctum (Labiateae),* rhizomes of *Zingiber officinale (Zingiberaceae),* fruits of *Piper longum (Piperaceae),* roots of *Glycyrrhiza glabra (Leguminosae)* and roots of *Withenia somnifera (Solanaceae)* were investigated for their antitussive effect on citric acid induced cough model in guinea pig. The results showed that the formulated cough syrup exhibited significant antitussive activity in a dose dependent manner the activity was compared with the prototype antitussive agent diphenhydramine HCl. It has been observed that the extract has produced 54%, 70%, 75% reduction in cough bouts at the dose level of 1, 2, 3 ml respectively after 1hr of drug administration. It is evident from the data the highest dose of 3 ml was found to be more effective. It is found that antitussive activity produced by the herbal formulation in the minimum dose was much better than the standard drug.

Keys words: Antitussive agent, Polyherbal cough syrup, Diphenhydramine HCl, Citric acid induced.

INTRODUCTION

Cough is a defensive reflex of the respiratory tract which is important to clear the upper airways and should not be suppressed indiscriminately. Cough is thought to be caused by a reflex. It occurs due to stimulation of mechano-or chemoreceptor in throat, respiratory passage or stretch receptor in the lungs. The sensitive receptors are located in the bronchial tree, particularly in the junction of the trachea. These receptors can be stimulated mechanically or chemically e.g. by inhalation of various irritants than nerve impulses activate the cough center in the brain. Traditionally cough is classified as either productive, *i.e.* producing mucus usually with expectoration or nonproductive (dry). Therefore, the use of an effective antitussive agent such as dextromethorphan or codeine to suppress the debilitating cough suffered by such patients seems appropriate. Non-Narcotic antitussive agents anesthetize the stretch receptor located in respiratory passages, lungs and pleura by dampening their activity and thereby reducing the cough reflex at its source. Narcotic antitussive agents depress the cough center that is located in the medulla, thereby raising its threshold for incoming cough[5]. *Terminalia chebula, Mentha piperita, Adhatoda vasica, Ocimum sanctum, Zingiber officinale, Piper longum, Glyzyrrhiza glabra* and *Withenia somnifera* are Perennial herb found throughout India also cultivated in some parts of Odisha. Fruits of *Terminalia chebula* contain chebuliagic acid, chebulinic acid, corilaogin & ellagitannin, *Mentha piperita* contain menthol, leaves of *Adhatoda vasica* contain vasicine, vasicinone, 6-hydroxy vasicine.and adhatodic acid and commonly used as expectorant and bronchodilator[6]. Leaves of *Ocimum sanctum* are contain 70% eugenol, carvacrol 3% and eugenol-methyl ether 20% and used as stimulant, aromatic, spasmolytic, stomachic and is a good immune-modulatory agent[7]. Rhizomes of *Zingiber officinale* contain geranial and citral, zingirone, paradols, ginediols and used as stimulant, aromatic stomachic[8]. Fruits of *Piper longum* contain l-phellandrene and caryophyllene and used to treat respiratory infection such as stomachic, bronchitis, diseases of spleen, cough, tumour and asthma[9]. Root of *Glyzyrrhiza glabra* contain glycyrrhizic acid and traditionally used as expectorant, demulscent and as flavouring agent in formulation with nauseous drugs like ammonium chloride, quinine, cascara[10]. Roots of *Withenia somnifera* contain withanine and used as respiratory stimulant, and as an immune-modulatory agent[11]. All these are used in skin eruption, skin infection, improve complexion and in cough. An attempt to prepare a poly herbal formulation were undertaken in the present study, after reviewing various literatures for each of these promising plants with a fore mentioned activities.

MATERIALS AND METHOD

Collection of plant materials

The fruits of *T. chebula, leaves of M. piperita, A. vasica, O. sanctum* and the Root of *G. glabra and W. somnifera* were collected from adjoining area of Barpali (Bargarh) in the month of February-2012. Fruits of *P. nigrum* and Rhizomes of *Z. officinale.* were purchased from the local market, Barpali, India and were authenticated from department of Botany, Panchayat College Bargarh, India .

Preparation of extracts

The collected plant materials (2 kg) of each were dried under shade, size reduced into coarse powder and macerated separately with 4 L of water-ethanol mixture (1:1). After 7 days of maceration, all the extract was filtered out and concentrated under vacuum using rotary vacuum evaporator (Sunilson et al., 2008)[12]. The residue obtained was kept in a dessicator for further studies.

Preparation of herbal syrup

The simple syrup (66.67% w/v) was prepared as per Indian pharmacopoeia. 200 mg of each extracts of *Terminalia chebula , Mentha piperita, A. vasica, Zingiber officinale.* and 400 mg of each extracts of *O. sanctum, Glyzyrrhiza*

glabra, Withenia somnifera and *Piper longum,* Honey were dissolved in simple syrup I.P. and the volume was made up to 100 ml and finally preservatives was added. [13]

Evaluation of formulated cough syrup

Physicochemical parameters like Specific gravity, Density, pH, Refractive index, Alcohol content and Acid value were analyzed as per the standard procedure mentioned in Indian Pharmacopoeia. The colour, odour and taste were also recorded[13] as shown in table -1.

Table 1: Physicochemical parameters of formulated polyherbal cough syrup

Colour	Reddish brown
Odour	Sweet aromatic
Taste	Sweet
Specific Gravity	1.25
Density	1.37
Refractive index	1.54
pH	4.8
Alcohol content	0.81
Acid value	0.118

PHARMACOLOGICAL SCREENING

Animals

Thirty healthy guinea pigs (300 - 400 g) of either sex were selected from the animal house of TPC, Barpali. They were kept in the departmental animal house under the conditions of light (14h light/10h dark) at 27 ± 2°C and relative humidity 44- 56%, for 1 week before and during the experiments. They were fed with standard diet and water was allowed to have *ad libitum.* All animals were handled according to the approval and current guidelines of Institutional Animal Ethical Committee[1].

Antitussive activity

The method described by (D. Marina et al 2008)[14] was adopted to evaluate antitussive activity.

The animal were divided into five groups of six each

Group- **I** control group

Group -**II** received diphenhydramine hydrochloride (2.8mg/kg)

Group -**III** received 1ml formulated cough syrup

Group -**IV** received 2 ml formulated cough syrup

Group -**V** received 3 ml formulated cough syrup

The animals were placed in a cylindrical glass vessel with two tubes at either ends. One serves as the entrance of the aerosol and the other for its efflux. The latter tube has a side arm connecting to a tambour, from which change in pressure can be registered. A pinch clamp with a variable screw was placed on the efflux tube beyond the side arm permitting the regulation of sensitivity of system so that the displacement of air in the enclosure caused by coughing of the animal was registered. The guinea pig was exposed to the aerosol of 7.5% citric acid in water for 10 min. Each animal was tested first to obtain the control response. The number of tussive response was registered. One hour later, the standard and test substances were applied orally and 30 min later the guinea pig was subjected to the aerosol again. The number of coughs during 10 min was recorded (Braga et al 1993)[15] (vogel et al 2002)[16, 17, 18].

Statistical analysis

All the data are expressed as mean ± SEM. The values obtained for the above parameters were compared with standard and control group using one way ANOVA followed by Student's test The values of $p < 0.05$ and $p < 0.001$ were considered to indicate a significant difference between the groups.

RESULTS

Aerosol of 7.5% citric acid in water produced tussive responses on guinea pig ileum. Herbal formulation in the concentration range of 1, 2, 3 ml revealed significant $p < 0.05$ and $p < 0.001$ antitussive activity in a dose dependent manner in comparision to control and standard drug. It has been observed that the extract has produced 54%, 70%, 75% reduction in cough bouts at the dose level of 1, 2, 3 ml respectively after 1hr of drug administration. It is evident from the data the highest dose of 3 ml was found to be more effective. Also the antitussive activity produced by the herbal formulation in the minimum dose was much better than the standard drug. The results were tabulated in Table -2, Figure - 1.

Table 2: Effect of polyherbal cough syrup on citric acid induced cough in guinea pig and treatment latency to citric acid induced cough

Treatment	**Latency to citric acid induced cough**		
Groups	Before in min (mean ± SEM)	After in min (mean ± SEM)	% reduction in cough bout against control
Control	16.1 ±2.79	13.3±1.79	-
Standard Diphenhydramine hydrochloride (2.8mg/kg)	20.8 ±1.21	8.83 ±1.45	45%
Test-I Formulated cough syrup (1ml)	17.8 ±1.97	7.33± 1.49**	54%
Test-II Formulated cough syrup (2ml)	20 ±2.89	4.48 ±1.05**	70%
Test-III Formulated cough syrup (3ml)	19 ±2.94	4.00 ±0.81**	75%

***P<0.01, P<0.05 shows statistical significance compared to control, n=6*

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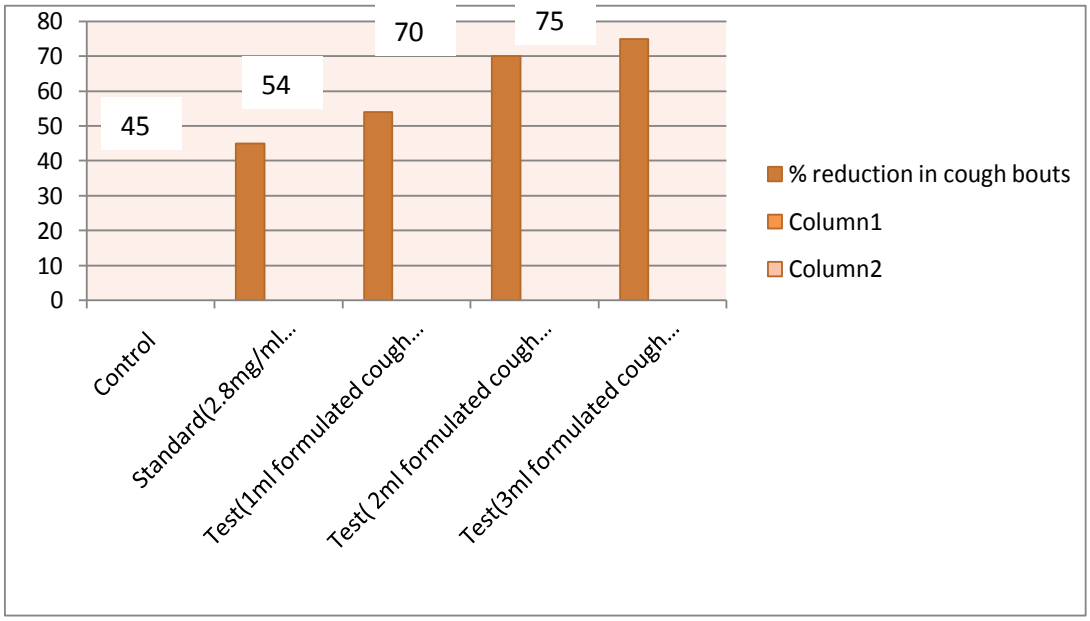

Figure 1: showing % reduction in cough bouts

DISCUSSION

Although, a number of synthetic preparations have proved to be effective for managing coughing symptoms, acurative therapy for cough is lacking. The cough suppressant activity elicited by the formulated herbal syrup may also be attributed to the presence of some phyto constituents such as vasicinone and vasicinol[1], Glycyrrhizin[9], eugenol, carvacrol, geranial, citral ,caryophyllene and withanine in the extracts of *Terminalia chebula, Mentha piperita, Adhatoda vasica, Ocimum sanctum, Zingiber officinale, Piper longum, Glycyrrhiza glabra* and *Withenia somnifera* respectively. Many of the currently available cough suppressants like Codeine, Ephedrine, Bromohexine, Guaifensesin etc, produce significant depression, drowsiness and addiction which makes their use unsatisfactory[9].

CONCLUSION

The present study has provided an experimental evidence for protection against cough by the formulated poly herbal cough syrup. All the above findings support the traditional claims in Ayurveda and Siddha for use of this formulation in the treatment of cough by virtue of its antitussive activity[19]. The Cough suppressant activity elicited by the formulated herbal syrup may also be attributed to the presence of some phyto constituents such as *T. chebula, leaves of M. piperita, A. vasica, O. sanctum, G. glabra, W. somnifera, P. nigrum* and *Z. officinale*. It can be concluded that the formulated polyherbal cough syrup in 1ml exerts a significant antitussive effect in experimentally induced cough reflex in mice comparable to the standard drug Diphenhydramine hydrochloride . The cough supressant actualy of polyherbal cough syrup was as compared to the actually of Diphenhydramine hydrochloride. The cough supressant activity in 3ml was 75% as compared to the actually of Diphenhydramine hydrochloride. The difference between test drugs and control group was very significant at the level of $p<0.01$.And the difference between test drugs and standard group (Diphenhydramine hydrochloride) was significant at the level of $p<0.05$.This may be suggestive of an antitussive activity of the formulation probably due to directly acting peripherally or may be due to Centrally.

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CONFLICTS OF INTEREST:

There is no conflict of interest in relation to the publication on manuscript file.

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